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Evalution of Anti-oxidants and Toxicological Activities of Crude Extracts from Argemone Maxicana

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ABSTRACT

The various metabolic reactions occurred in the body of organisms produce oxidants or free radicals. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electron and involved in many pathological conditions. The various secondary metabolites produced by plants may act as anti-oxidant by scavenging these free radicals. The extract was screened for anti-oxidant activity by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method.

It has been find out that the IC_{50} values obtained for DPPH inhibition of two column fractions were 105.443µg/ml, 394.564 µg/ml and 251.57 µg/ml for first fraction (F1) i.e methanolic fraction, second fraction (F2) i.e. ethyl acetate, and ascorbic acid respectively. Help of uv-vis spectrometer is taken to calculate the percentage of anti-radical activity by taking absorbance.

Keywords: Anti-oxidants, toxicological activities, argemone maxicana.

1. INTRODUCTION

Antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances from different plant sources. The natural products derived from plants were being tested for presence of new drugs with new modes of pharmacological action. Also, there is a need to develop

alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants or a constant need for new and effective therapeutic agents.

In developing countries, infectious diseases remain the main cause of the high mortality rates recorded; the majority of rural people has limited access to formal and adequate health services and thus heavily resources to traditional healers Many contaminants and hepatotoxic drugs are considered to be reason responsible for causing hepatic damage including viral infection. In most of the cases these hepatotoxicity is mediated through free radicals. This free radical causes oxidative stress in hepatic tissue, which is responsible for variations on normal physiology of liver. Herbs are always considered to be an important source of medicine. They are used by traditional healers for treatment of various ailments including hepatic disorder. Herbs are also having good potential to work as antioxidant agents.

2. METHOD

Determination of antioxidant activity of methanolic and Ethyl acetate fractions (F-1 and F-2)

The fractions F-1 and F_{-2} obtained through the course of column chromatography of methanol and ethyl acetate crude extact were subjected to antioxidant activity to find the most potent fraction.

The method used for the determination of scavenging activity of DPPH free radical in the extract solution is as follows. A solution of 0.135mM DPPH in methanol was prepared and 1.0ml of this solution was mixed with 1.0ml of extract prepared in methanol containing 0.025-0.5mg and standard drugs separately (Gallic acid). The reaction mixture was vortex thoroughly and left in the dark at room temperature for 30min. the absorbance of the mixture was measured spectrophotometrically at 517nm.

The ability of plant extract to scavenge DPPH radical was calculated by the equation

$$\% \text{ anti-radical activity} = \frac{\text{control absorbance} - \text{sample absorbance X } 100}{\text{Control absorbance}}$$

3. RESULTS

Hydrogen peroxide scavenging activity for the Ethyl acetate extract was performed by using Ascorbic acid solution as standard. The Ethyl acetate extract and ascorbic acid showed prominent IC_{50} values of 1213.58µg/ml and 4.47 µg/ml respectively.

Table 1. Showing Antioxidant activity of F-2, Ethyl acetate extract by H_2O_2 method

S. No.	Concentration µg/ml	% scavenging activity	**IC_{50}**
1	100	7.36	
2	200	15.03	
3	400	24.41	1213.58
4	600	26.74	
5	800	35.27	
	Control	0.678	

Table 2. Showing Antioxidant activity of ascorbic acid by H_2O_2 method

S. No	Concentration (µg/ml)	% Inhibition	IC_{50}
1	25	51.94611	4.47 mg/ml
2	50	54.64072	
3	100	57.48503	
4	150	60.92814	
5	200	67.96407	

Table 3.Showing Antioxidant activity of F-1, methanolic extract by DPPH method

S. No	Concentration (µg/ml)	% Inhibition	IC_{50}µg/ ml
1	25	18.43	105.443
2	50	24.53	
3	100	47.57	
4	150	69.47	
5	200	89.46	

Table 4. Showing Antioxidant activity of ascorbic acid by DPPH method

S. No	Concentration (µg/ml)	% Inhibition	IC_{50}
1	25	30.75746	251.57 µg/ml
2	50	37.49044	
3	100	39.3267	
4	150	43.07575	
5	200	45.37108	

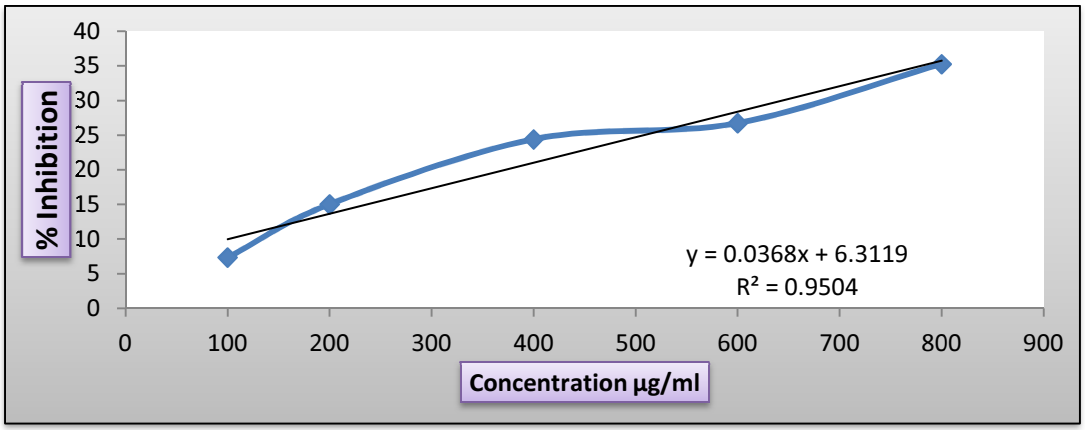

Figure 1 Showing Antioxidant activity of extract by H_2O_2

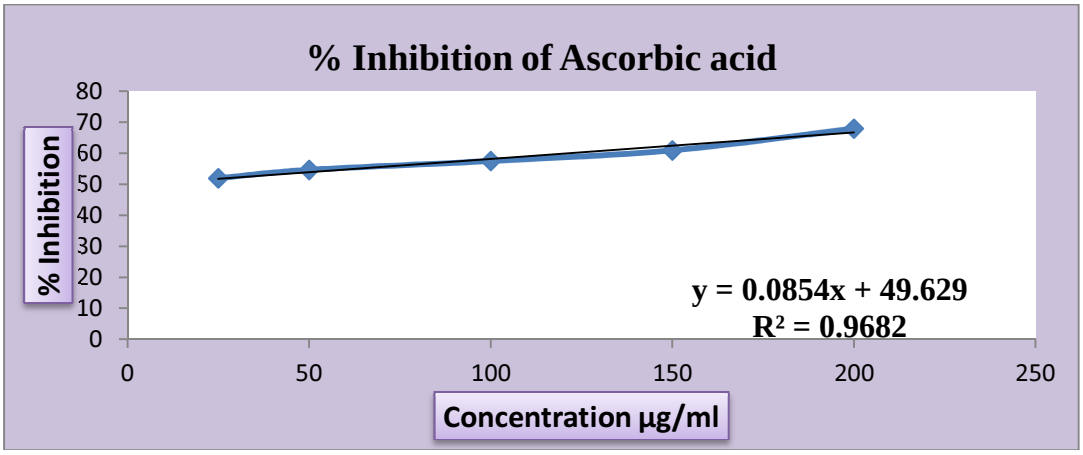

Figure 2 Showing Antioxidant activity of ascorbic acid by H_2O_2 method

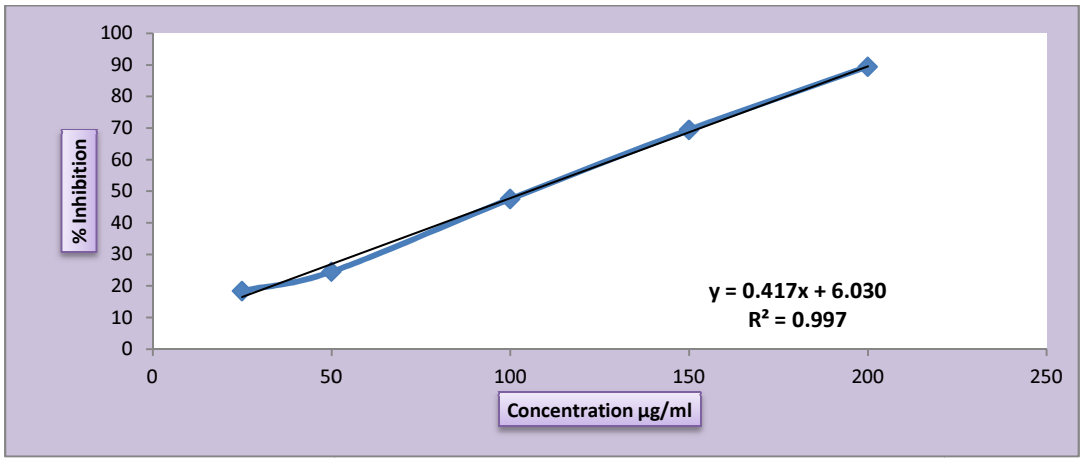

Figure 3 Showing Antioxidant activity of extract by DPPH method

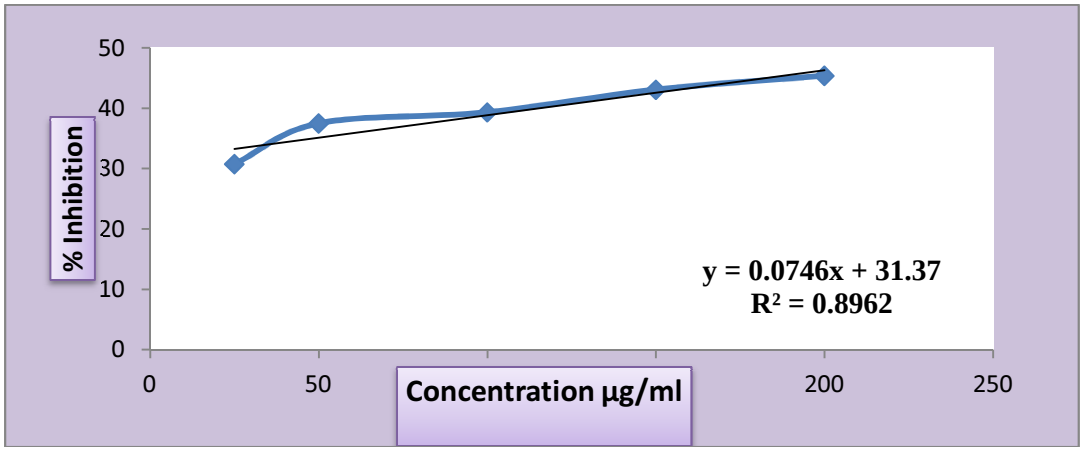

Figure 4 Showing Antioxidant activity of ascorbic acid by DPPH method

The results revealed that fractions F1 are having higher percentage inhibition of DPPH absorbance and lower IC_{50} values of 105.443µg/ml when compared to IC_{50} value of ascorbic acid (251.57µg/ml). It was shown from above mentioned results that fraction (F-1) methanol, has higher antioxidant activity between the two fractions.

4. DISCUSSION

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once inside the cell, H_2O_2 can probably react with Fe2+and possiblyCu^{2+} hydroxyl

radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. In hydrogen peroxide assay it was observed that extract showed linearity in percentage inhibition in concentration range selected, with good goodness of fit (R^2=0.950) and hence using the curve IC50 of test sample was found to be 1213.58µg/ml. This was found to be quite less as compared to ascorbic acid in which IC 50 was found to be 4.47 µg/ml.

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge (Eklund *et al*., 2005). Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay (Alma,2003) 1,1-Diphenyl-2-picrylhydrazyl (DPPH), is a kind of stable organic radical. The DPPH oxidative assay (Peng, *et al.*, 2000) adopted in the paper is used worldwide in the quantification of radical-scavenging capacity (RSC). The capacity of biological reagents to scavenge the DPPH radical can be expressed as its magnitude of antioxidation ability. The DPPH alcohol solution is deep purple in colour with an absorption peak at 517 nm, which disappears with the presence of the radical scavenger in the reactive system and when the odd electron of the nitrogen in the DPPH is paired. The reactive rate and the ability of the radical scavenger depend on the rate and the peak value of disappearance of the DPPH (Chi, 2003). Compared with other methods, the DPPH assay has many advantages, such as good stability, credible sensitivity, simplicity and feasibility (Ozcelik, *et al*., 2003). In present investigation antioxidant potential of extract was also investigated using DPPH assay. IC 50 for DPPH was found to be 105.443 µg/ml. In selected concentration range of 25 to 200 µg/ml there was linearity in percentage inhibition with R^2 = 0.997.

The best-described property of almost every group of flavonoids is their capacity to acts as antioxidants. In humans, flavonoids undergo intracellular metabolism, e.g., conjugation with glutathione, and circulating flavonoids are usually *O*-methylated or glucuronidated (Rodrigues, 2011). In present investigation total flavonoid content in extract was estimated using Rutin a well established flavonoid. Standard curve Rutin was prepared and using the line of regression of Rutin y= 0.002x - 0.002, R^2 = 0.964 it was observed that total flavonoid content (TFC) was 104.08±33µg/ml. TFC was expressed as Rutin equivalent (RE).

5. REFERENCES

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